REMARKS

Claims 245-255, 258, 262 and 265 are pending in the above-referenced application; claims 268 and 269 have been withdrawn from consideration. As will be discussed in further detail below, claims 245, 255 and 262 have been amended to more distinctly claim that which Applicants regard as their invention. The claim amendments are supported by the specification. Specific support will be discussed in pages 25-28 of this response. No new matter has been added. Furthermore, claims 247 and 252 have been amended to correct their dependencies.

I. SUBSTANCE OF INTERVIEW

Applicants wish to thank Examiners Hudson and Angell for their time and helpful suggestions during their telephonic interview with the undersigned, the Examiner's representative, Cheryl H. Agris and one of the inventors, Dr. James Donegan on May 1, 2008. The substance of the interview is discussed below.

A. Brief Description of any Exhibit Shown or any Demonstration Conducted

Applicants submitted pages 81-90, 145-146, 154-157 and Figures 24-25 of the specification since these pages and figures were referred to during the interview. Additionally, Applicants submitted Balvay et al., 1993, BioEssays 15:165-169 ("Balvay"); Johansen et al., 1996, Proc. Natl. Acad. Sci. USA 93:12400-12405 ("Johansen"); Mayeda and Oshima, 1990, Nucl. Acids Res. 18: 4671-4676 ("Mayeda and Oshima") and Yoshimatsu et al., 1989, Science 244:1346-1347 ("Yoshimatsu").

B. Identification of Claims Discussed

Claims 245, 255 and 262 were discussed.

C. Identification of Specific Prior Art Discussed

As will be set forth in further detail below, Balvay, Johansen, Mayeda and Oshima, and Yoshimatsu were discussed with respect to the rejections under 35 USC §112.

D. Identification of Principal Proposed Amendments of a Substantive Nature Discussed

Amendments to claims 245, 255 and 262 were discussed.

E. Identification of General Thrust of Principal Arguments presented to the examiner

An adequate description and enabling disclosure has been provided to support the pending claims. No new matter is contained in the pending claims.

F. A General Indication of Any other Pertinent Matters Discussed

No other pertinent matters were discussed.

G. General Results or Outcome of the Interview

Applicants agreed to submit arguments to support assertions of adequate written description and enablement. Applicants agreed to point out with specificity sections of the specification that support the instant claims.

II. The Rejection Under 35 USC 112, First Paragraph (Written Description)

Claims 245-255, 258, 262 and 265 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. Before responding to the rejection, Applicants note that claim 245 has been amended to recite that the claimed construct comprises a nucleic acid sequence which encodes a polymerase and comprises a recognition site for said polymerase; claims 255 and 262 have been amended to recite that when the claimed construct is in a eukaryotic cell, the intron is removed during processing and the gene product is expressed in a eukaryotic cell after removal of this intron. As will be discussed in further detail below, Applicants assert that there is adequate written description for subject matter recited in claims 245-255, 258, 262 and 265.

The Office Action specifically states:

> Applicant argues that the instant claims recite that the presence of the intron is responsible for lack of expression in a prokaryotic cell and therefore one would not use any

> intron. Applicant argues that the intron used would have to be responsible for lack of expression of the gene product in a prokaryotic cell. Applicant asserts that one of skill in the art would know which features are necessary in the intron in order to possess this function. Applicant explains that for example, the simple expedient of determining the number of nucleotides in the intron would generate a frame shift in the coding is an obvious approach which also indicates that 1/3 of the introns chosen at random would have this feature; the presence of stop codons is another easily ascertainable approach, and thus it would be evident to one of skill what characteristics in an intron would be appropriate to use the constructs of the invention.
>
> However, it is important to note that the instant claims are not limited to the embodiments addressed by applicant above. The specification does not provide support for the use of any intron, in any polymerase or any bacteriophage polymerase, or any conditionally toxic gene, in any eukaryotic or prokaryotic cell because the specification provides only minimal description of any particular intron, polymerase (including bacteriophage polymerase), or toxic gene, or eukaryotic or prokaryotic cells for whom known structures exist that could be utilized having the claimed function.

In response, Applicants first wish to clarify that 1/3 of the introns would actually be <u>in frame</u> after insertion of an intron and it would be the majority (2/3) of insertion events that would generate frameshift mutations in the coding sequence and inactivate a target gene. The fact that a frameshift mutation(s) and/or stop codons in a transcript will inactivate expression of a gene is well known in the art. Applicants have stated on page 86 of the specification:

> A preferred mode of inactivation is the use of a heterologous processing element that introduces a frame shift mutation and/or a stop codon(s).

It is Applicants' view that the presence of sequences in an intron that will contribute a frameshift mutation(s) and/or stop codons is an easily ascertainable property of a chosen intron candidate that will predict the likelihood of success for that sequence before conducting a single experiment. Applicants have disclosed the use of an SV40 intron in the Examples section. The SV40 intron contains stop codons in all three reading frames. However, in Applicants' view, this is not a special

feature of this intron. Statistical analysis shows that since there are three possible stop codons, UAA, UAG, and UGA, there is a 3 in 64 chance of any random group of three nucleotides being a stop codon. In a 200 bp intron (which would be considered to be a small intron), there would be 66 possible triplets in each reading frame, which means that on average there would be three stop codons present in each reading frame for a 200 bp random sequence. Thus, the presence of so many stop codons in SV40 should be considered to be a typical property. It should be pointed out again that one of the hallmarks that identified the presence of introns in early studies was the presence of stop codons in all three reading frames, or to put it another way the absence of long ORF (Open Reading Frames) sequences. As an example, reference to an intron inserted into a coding sequence was described on page 4 of the specification (Schwartz et al., 1993, Gene 127:233, hereinafter "Schwartz" and attached hereto as Exhibit 1) where disruption of expression was endowed by a frameshift mutation due to the insertion as well as the presence of two in-frame stop codons (see Schwartz, p. 234). As such, selection of an intron sequence whose insertion would inactivate a selected gene transcript would be easily ascertainable to one skilled in the art.

Applicants would next like to respond to the assertion in the Office Action that "the specification does not provide support for the use of any intron, in any polymerase or any bacteriophage polymerase, or any conditionally toxic gene, in any eukaryotic or prokaryotic cell". With reference to "any polymerase", there is support for the use of any polymerase chosen by the user as long as its sequence is known. Numerous polymerases besides T3, T7 and SP6 were known as of the priority date of the above-referenced application that theoretically could also have been used with the present invention. Rules for the selection of a site within a target sequence, (C/A)AGG) are given on pages 84 and 146 of the specification and should be universally applicable to any target protein. The recitation in the claims of conditional inactivation by insertion of an intron ("incapable of being expressed in a prokaryotic cell due to the presence of said intron") is sufficiently limited that the teachings disclosed in the specification will allow the user to practice the present invention.

Furthermore, claims 255 and 262 recite that the gene product is expressed after the removal of the intron.

With reference to "any intron", as discussed in the specification, a wide variety of introns had been sequenced by 1982 that allowed the description of the consensus sequence of splice donors and acceptors (Mount, 1982, Nucl. Acids Res. 10:459, hereinafter "Mount"). An obviously larger number were subsequently described between the Mount paper and the 1995 priority date of the instant application. Thus, there was a large body of knowledge available to the user concerning intron sequences at the time of the filing.

Applicants further disagree with the assertion that there is no support for any conditionally toxic gene. Applicants are describing a novel method that applies a basic piece of biological knowledge: disruption of a gene by insertional mutagenesis. In the particular method that was disclosed, the mutational insertion event allows excision of the extraneous sequences in a eukaryotic cell, thereby allowing phenotypically "reversal" of the mutation in the proper cell. Secondly, there is a lengthy discussion in the specification of how an RNA polymerase coded by a plasmid that also contains its cognate promoter would be considered to be a toxic gene when the plasmid is present in a prokaryotic cell. Descriptions of other toxic genes that would benefit from conditional inactivation are given on page 87 of the specification:

> Thus genes which would be impossible to clone, such as those which code for enzymes which destroy bacterial cell walls, can be inactivated by intron insertion and thus cloned in this form in a bacterium. Genes coding for toxic products, including tetanus toxin, ricin, pseudomonas toxin, E. coli enterotoxins, cholera toxin and other plant, animal, and microbial toxins, can be inactivated and maintained stably and safely in incompatible cells and activated to produce an unaltered gene product in a compatible cell.

Thus, "toxic" is given the meaning and exemplified as not only being toxic to a host cell (as exemplified by the T7 plasmid discussed above as well as bactericidal proteins) but also describes proteins which are intrinsically toxic whereby the cloning and maintenance of constructs coding for such products will be safer where the toxic

product coding sequence remains in an interrupted state until the product is actually desired. Thus, an adequate description of "toxic" was provided.

With regard to eukaryotic and prokaryotic cells, no other features are required to be known about them since it is a well studied phenomenon that eukaryotic cells universally provide machinery for splicing out introns and prokaryotic cells universally lack such machinery. Thus, an adequate description is provided for any prokaryotic or eukaryotic cells.

The Office Action further states:

> The specification provides for the use of T3, T7 or SP6 polymerases, and also for the use of certain "consensus" splice donor and acceptor sites for inserting introns. Applicants prophetically suggest that intron "insertion at any of these sites in a gene coding region should not affect subsequent removal of the processing element in a compatible cell." (page 84 of the instant specification). However, there is significant unpredictability in such intron removal, since such a process requires a complex interaction between the nucleic acid construct and the already existent cellular machinery.

In response, Applicants wish to clarify that the (C/A)AGG sites in the target genes are not consensus splice donor and acceptor sites but rather these sites resemble a post-splice site. In other papers that have been published concerning intron development, this sequence is described as a proto-splice site (see, for example, Dibb, 1993,FEBS Lett. 325:135-139, attached hereto as Exhibit 2). The specification teaches that the convenience of these sites are that the sequences will be converted into splice donor and acceptor sites by the addition of the flanking intron sequence. As the Balvay reference notes, the splice donor site consensus sequence is (C/A) AG-GU(A/G)AGU. By inserting an intron between the last two G's of the (C/A)AGG sites described in the specification, these sites are converted into the consensus (C/A) AG-GU(A/G)AGU sequence (which is complementary to an 8 base sequence in U1 RNA) where the first three nucleotides are contributed by the target gene (exon sequences) and the last 6 nucleotides are contributed by the intron. A similar situation exists in the 3' acceptor site where the majority of the sequences are derived from the intron itself. Applicants do disagree with the

assertion that there is significant unpredictability in intron removal since the teachings of the specification as well as knowledge in prior art allow the user to generate splicing sequences that are likely to carry out splicing events of a known and predictable nature.

On page 4 of the Office Action, it is stated:

> Furthermore, applicant asserts that the methods used to block expression are not related to the ultimate function of the protein and therefore the only knowledge necessary would be the sequence of the protein or polymerase so that an appropriate site could be chosen. However, the instant specification does not describe such a broad genus of nucleic acid constructs that would conditionally control the expression of any polymerase or protein sequence based on the presence of any intron in any eukaryotic or prokaryotic cell. The specification does not disclose a structural characteristic that would allow one of ordinary skill to recognize which introns introduced into which sequences would result in expression or lack of expression of which polymerases or proteins.
>
> Contrary to applicant's assertions, the specific example given in the specification is not representative of the broad genus of nucleic acid constructs that are instantly being claimed. The structural characteristics recited in the instant claims are extremely broad and the specification does not disclose a structural characteristic that would allow for the skilled artisan to envisage the entire genus claimed of nucleic acid constructs with any intron that would result in any polymerase to be incapable of being expressed in any prokaryotic cells and capable of producing a nucleic acid sequence when introduced into any eukaryotic cell. Therefore, the skilled artisan would not be able to recognize that applicant was in possession of such a broad genus of nucleic acid constructs at the time of filing.

In response, Applicants assert that one skilled in the art is fully capable of recognizing the characteristics that would allow a user to choose a particular intron (stop codons and induction of frameshift mutations) as discussed above. The particular intron disclosed in Example 19, the SV40 intron, was an example of a wide variety of introns that would be understood to be of use in the present invention.

Therefore, in Applicants' view, the specific example given was indeed representative of the broad genus of nucleic acid constructs claimed.

The Office Action in the written description rejection again cites Balvay:

> Applicant asserts that the Balvay et al. reference is better suited for a post-hoc explanation of why a particular site did not work rather than a predictive tool of why something is unlikely to work and that Balvay et al. only concerns itself with natural splice sites in natural genes. Contrary to applicant's assertions, the teachings of Balvay et al. are highly relevant to the breadth of the instant claims, as Balvay et al. discusses splicing machinery. Balvay et al. indicates that the splicing machinery is highly dependent upon recognizing and interacting with such secondary structures in making the splice. Balvay et al. indicates that the addition of a secondary structure to an existing mRNA can cause the cell to splice at a point not normally spliced at, while removal of such a structure can cause splicing to be eliminated (for example see pages 165 bridging to 166).
>
> Furthermore, Balvay indicates that the exon plays a significant role in splice site recognition by the cellular splicing machinery. Since one of skill would understand that the nucleotides in the exon remain in the mRNA (or ribozyme) after splicing, applicants claimed nucleic acid constructs, following splicing, would likely therefore contain elements of these exon recognition sites. Such unpredictability indicates that the genus of nucleic acid constructs comprising any intron in any polymerase (or any bacteriophage polymerase), or any toxic gene, and that are active or inactive depending on whether they are found in prokaryotic or eukaryotic cells is very large…..
>
> …Although applicant asserts that the examples that Balvay bring up are more in the nature of exceptions to the general rule and that there are normal rules of how and where splicing would occur and are predictable, these are simply assertions that are not supported by the instant specification or the art. The teachings of Balvay et al. support the unpredictability of the splicing mechanism, rather than the presence of common knowledge of the skilled artisan to the contrary, as asserted by applicant.

Applicants disagree with the assertions made in the Office Action that Balvay was **not** concerned with exceptions. The first sequence analyzed by Balvay was characterized on page 165 as follows: First the title of the section was "Secondary

structure can allow selection of an **abnormally** located 3' splice site" and then later "One of the **peculiarities** of this reaction……. (emphasis added). Thus, this particular splicing event is described as both "abnormal" and "peculiar". The use of the term "abnormally located" has a definite implication that a different site would have been expected to be used for the spicing and therefore such sites would be considered to be predictable under most circumstances, i.e. this situation could be recognized as an exception to standard expectations. Furthermore, it should be noted that the particular abnormality that is exhibited in this intron has to deal with secondary structures within the intron and not with flanking exon sites; i.e. this is a peculiarity intrinsic to the intron itself. Presumably insertion of this intron to another location would also carry along its peculiarities with it and thus even as an exception it would remain predictable.

The next case that was cited in Balvay (page 166) was under the title heading "3' splice site selection and the **rule** of the first come the first served". When discussing this case it was characterized as "a rather **unusual** situation" and deals with the concept that in contrast to the usual "first come, first served" rule (the first potential acceptor site downstream is used), an apparently normal acceptor site is ignored and it is the next one downstream that is used. Their explanation of the reason for this situation is summarized at the end of the section as "and also explains some **exceptions** to the scanning mechanism which is supposed to control the choice of 3' splice site (the "first come, first served" rule mentioned above)". It is again to be noted that the change in what are acknowledged to be "rules" is dictated by a secondary structure formation within the intron. In this particular instance, a person skilled in the art would recognize that the intron described in this section would span from the 5' donor to the second potential acceptor site (the one that is actually utilized) and insertion of this unit would retain these properties and thus even this exceptional situation would remain predictable.

The next example given in Balvay is on page 167 referring to an E3 cryptic donor site and the influence of secondary structure on its use. In this particular case, it is never said that the expected donor site would not be used but rather in cases of mutations that disrupt a proposed secondary structure in the exon, there is an

additional use of the cryptic site while the normal donor site continues to be used as well. Thus, this intron would also be expected to generate a predictable product.

Applicants would like to point out in general that the discussion of pages 165-167 of Balvay et al. is couched in terms of differences in what would be expected from what was known at the time in terms of splice site selections. Without a set of rules that were considered to be generally predictive of splicing events, there would never be "expectations" as well as exceptions and abnormality that disagreed with such expectations. As such, all of the preceding cases describe exceptions to these rules and the presence of appropriate splice consensus sequences was considered to be a predictable expectation of the use of such sites such that when they are not used in such a manner, the cited cases were considered to be unusual, exceptional or abnormal situations. Applicants for Examiner's reference attach a copy of Balvay (Exhibit 3) where pertinent passages are highlighted.

In summary, splicing events can only be considered to be abnormal or unusual when there already exists a normal or usual expectation of events, i.e they are predictable events where certain cases did not match the prediction. As stated above, the terms "abnormally", "peculiarities", "unusual", "exceptions" are words that Balvay used to describe these situations and are not assertions interpolated by the Applicants. Thus, for the reasons set forth above, Applicants do indeed take exception to the assertion made on page 6 of the Office Action that

> Balvay bring up are more in the nature of exceptions to the general rule and that there are normal rules of how and when splicing would occur and are predictable, these are simply assertions that are not supported by the instant application or the art.

Applicants, in contrast, wish to reinforce their view that Balvay did not stand for the premise that splicing was unpredictable but instead that splicing was governed by a set of rules but that there were exceptions.

In view of the above arguments, Applicants assert that the rejections under 35 USC 112, first paragraph (written description) have been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

III. The Rejection Under 35 USC 112, First Paragraph (Enablement)

Claims 245-255, 258, 262 and 265 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Office Action specifically states:

> Applicant asserts that Example 19 provides a more than sufficient description regarding strategies used in choosing intron sequences to be used, insertion sites in the T7 polymerase and vectors, as well as construction steps. It is noted that the specific example in Example 19 is not commensurate in scope with the broadly recited characteristics of the nucleic acid constructs of the instant claims and does not reasonably provide predictability of such a broad genus of nucleic acid constructs having the instantly desired function.

Applicants disagree and still assert that Example 19 as well as the instant specification provides a more than sufficient description regarding strategies in choosing intron sequences. Example 19 discloses a T7 polymerase containing an SV40 intron. As noted in the previous section of this response, there were stop codons in all three reading frames of the SV40 intron. In Applicants' view, the presence of so many stop codons in SV40 should be considered to be a typical property. As also noted above, one of the hallmarks that identified the presence of introns in early studies was the presence of stop codons in all three reading frames, or to put it another way the absence of long ORF (Open Reading Frames) sequences. As such, selection of an intron sequence whose insertion would inactivate a selected gene transcript would be well known in the art.

The Office Action further states:

> Applicant asserts that methods are well known in the art for introducing artificial introns. It is not disputed that methods are known in the art to introduce artificial introns. However, it is the unpredictable nature of introducing any intron into any nucleic acid sequence at any position that encodes any polymerase with a resultant incapability of the polymerase being expressed in any prokaryotic cell, whereas more than one copy of a nucleic acid sequence is produced when introduced into any eukaryotic cell. Furthermore, the claims recite that the gene product or

> protein expressed would be toxic specifically to a prokaryotic cell in the absence of the intron.

In response, Applicants wish to clarify that claim 245 is directed to a construct encoding a polymerase. *Contra* to assertions made in the Office Action, it is not directed to just any polymerase. The polymerase recited in claim 245 must be (a) incapable of being expressed in a prokaryotic cell, due to the presence of said intron and (b) capable of producing more than one copy of a nucleic acid sequence from said construct when introduced into a eukaryotic cell. Further, the intron in claim 245 is non-native to said polymerase, wherein said intron sequence is within the sequence encoding said polymerase. Thus, the claims are not directed to just any intron. Applicants further point out that claim 252 is directed to a nucleic acid construct which comprises a sequence that encodes a gene product specifically toxic to a prokaryotic cell in the absence of a non-native intron. Thus, the construct further comprises an intron sequence non-native to said gene product. Applicants note that claims 245 and 252 are independent claims and are thus mutually exclusive. A polymerase is not necessarily toxic.

The Office Action further states:

> Applicants' specifically claim that the inserted and inactivating intronic sequences will be spliced out, a process the specification indicates will be carried out by the cellular machinery that normally operates to splice introns out of pre-mRNA sequences. Applicants indicate that such splicing restores native activity to previously inactive proteins. However, the specification as filed does not provide any nucleic acid constructs for which this has actually been shown to demonstrate the predictability of such a broad mechanism. Applicant's specification does not provide sufficient guidance or examples that would enable a skilled artisan to make the disclosed nucleic acid constructs containing sequences that are spliced out by cellular machinery without undue experimentation. Although the specification prophetically considers and discloses making and using such constructs, such a disclosure would not be considered enabling since introducing intervening sequences into nucleic acids alters their secondary structure, which makes their ability to be cleaved by the splicing machinery unpredictable. The

> specification has not resolved such issues, since no exemplified constructs that contain intervening sequences and are inactive therefore, and by which later processing inside the cell restores activity. Applicants have simply not shown that such intervening sequences can be spliced out to restore any activity to previously inactive polymerases (or any toxic protein for that matter).

Applicants disagree. As set forth in the previous response filed and as discussed during the Interview on May 1, 2008, the instant specification combined with knowledge of one skilled in the art would enable a skilled artisan to make the disclosed and claimed nucleic acid constructs containing sequences that are spliced out by cellular machinery. As noted above, on page 4 of the specification, reference was made to Schwartz where an intron was inserted into the coding sequence of the neo gene resulting in a lack of expression in bacterial cells that was reconstituted in mouse and hamster cells. The predicted splicing events in the intron modified mRNA were verified by isolation of cDNA followed by sequencing. Further, the specification, on page 85 of the specification makes reference to a broader study by Mayeda and Oshima as support for the statement "Furthermore, the site of insertion for a processing element does not appear to affect gene expression" describing incorporation of a globin intron into **various** sites which would then be spliced out correctly. Specifically, seven distinct positions in the globin gene were used by Mayeda and Oshima and led to the conclusions:

> These results suggested that **only** 3 nt (CAG) of the specific 5' exon sequences are required and that **the position of an intron has no significant effect on its removal**. Mayeda and Oshima at 4672.

> The results presented here show that when the intact intron is flanked by three conserved nucleotides (CAG) of 5' exon, the intron is **always** efficiently spliced out **regardless of surrounding exon sequences or position**, except when the 5' or 3' exon is too short. *Id* at 4673.

> We showed that **the cis-acting determinants essential for pre-mRNA splicing are localized in the intron itself plus 3 nt of the 5' exon rather than in the overall structure of pre-mRNA**." (emphasis added). *Id* at 4676.

Thus *contra* to assertions made in the Office Action that insertion of an intron sequence into mRNA gives unpredictable results, Mayeda and Oshima have described a practical exercise that demonstrated insertion of an intron into novel sites resulted in the predicted splicing out of the intron. This pragmatic demonstration of an exercise that parallels the teachings of the present invention should have been sufficient proof that intron sequences inserted into a target gene at (C/A)AGG proto-splice sites are likely to be spliced out correctly.

Further evidence that the level of predictability is high was seen in other publications as well. In the previous response filed, reference was made to Gatermann et al., 1989, Mol. Cell Biol. 9:1526-1535 ("Gatermann 1989) and Yoshimatsu and Nagawa, 1989 Science 244; 1346-1348 ("Yoshimatsu and Nagawa 1989") where insertions of introns into varied sites were carried out and there was a lack of any descriptions of splicing events taking places at sites other than those expected and predicted. Statements from these citations were included in the last response as follows:

> "the proper signals with an intron are sufficient to initiate and complete splicing events independent of the location of the intron in the gene" (Gatermann 1989)
>
> "any gene can be converted to a controllable gene by the simple insertion of an intron" (Yoshimatsu and Nagawa 1989)

Success with intron insertion generating predictable results was continued in publications that came out after the filing as well. One example is Johansen, "Intron insertion facilitates amplification of cloned virus cDNA in *Escherichia coli* while biological activity is reestablished after transcription *in vivo*", 1996, Proc. Nat. Acad. Sci. (USA) 93:12,400-12,405, hereinafter "Johansen" attached hereto as Exhibit 4. A reading of the abstract is very informative:

> Insertion of introns into cloned cDNA of two isolates of the potyvirus pea seedborne mosaic virus facilitated plasmid amplification in *Escherichia coli*. Multiple stop codons in the inserted introns interrupted the open reading frame of the virus cDNA, thereby terminating undesired translation of virus proteins in *E. coli*. Plasmids containing the full-length virus sequences, placed under control of the

> cauliflower mosaic virus 35S promoter and the nopaline synthase termination signal, were stable and easy to amplify in *E. coli* if one or more introns were inserted into the virus sequence. These plasmids were infectious when inoculated mechanically into *Pisum sativum* leaves. Examination of the cDNA viruses confirmed that intron splicing of *in vivo* transcribed pre-mRNA had occurred as predicted, reestablishing the virus genome sequences. Symptom development and virus accumulation of the cDNA derived viruses and parental viruses were identical. It is proposed that intron insertion can be used to facilitate manipulation and amplification of cloned DNA fragments that are unstable in, or toxic to, E. coli. When transcribed *in vivo* in eukaryotic cells the introns will be eliminated from the sequence and will not interfere with further analysis of protein expression or virus infection.

Two different sites in one gene, three different sites in a second gene and a single site in a third target gene were all successfully tested with this method. In addition, three different introns were employed. In summary, there was a post-filing finding by Johansen of a lack of problems with the unpredictability described in the Office Action and success was consistently achieved for a variety of introns, in a variety of genes and in a variety of sites. It can be concluded from the Johansen reference that a person skilled in the art is on record of believing that a) the presence of stop codons in an inserted intron is a practical and desirable method of terminating expression of a target gene in *E. coli* b) that the intron can be predicted to be spliced out correctly and the target sequence reconstituted properly in cells that possess splicing machinery and c) that this method can be applied in general to toxic genes.

Thus in contrast to the statements made in the Office Action on pages 10 and 11, the state of the art at the time of the filing would have allowed a user to proceed with insertion of introns with reasonable confidence that even in the new locations, the introns would most likely be properly spliced out and there would not be undue experimentation involved in either the choice of the intron or the choice of the insertion site that would allow suppression of expression of the gene product in a bacterial cell but would allow expression of a functional product in a eukaryotic cell. The insertion could be carried out using methods that were described in the

specification or other methods that were described in the literature at the time of the filing could be employed.

Further reference is made to Balvay in the Office Action and reference is also made to Lewin:

> Applicant points to a statement of Balvay et al. "It is important to stress that in the absence of in vivo experiments or in vitro systems where transcription and splicing are coupled, all these conclusions about the functional significance of secondary structure should be taken as tentative ones." Although applicant interprets this statement as a tentative conclusion that is contrary to practical exercises that have been carried out generating in vivo data that introduction of introns into selected sites is a predictable art with a high likelihood of success, the statement of Balvay et al. actually supports the examiner's position. It is agreed that the issues of unpredictability due to secondary structure as taught by Balvay et al. could be overcome by in vivo experimentation, Balvay et al. is evidence that there are additional considerations such as secondary structure that would lead to unpredictability, absence evidence to the contrary. The instantly recited constructs have extremely broad structural characteristics that were not enabled by the instant specification or the state of the art at the time of filing.
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> Again, the issue is not whether it was known in the art how to insert introns, but rather how to insert introns in a predictable fashion in accordance with the breadth of the instant claims and have the desired outcome specific to eukaryotic and prokaryotic cells with regards to any polymerase, as recited in the instant claims. Balvay et al. is simply an example that secondary structure is one complexity when considering splicing mechanisms.
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> In particular, it is demonstrated that the complex secondary structures of nucleic acids are responsible for their intron excision activity, and furthermore, that predicting the ability of the cellular splicing machinery to splice out precise intervening sequences from disrupted sequences with variable secondary structures such that native activity is restored is considered unpredictable, because the splicing machinery is sensitive to the presence or absence of such structures.
>
> Applicant relies on Lewin for teachings regarding experiments of splicing out a hybrid intron and teachings

> that splicing sites are generic, meaning that they do not have specificity for individual RNA precursors and the RNA precursors do not convey specific information (such as secondary structure) that is needed for splicing. The teachings of Lewin et al. do not diminish the unpredictability of the intron splicing mechanism when a non-native intron is inserted into a sequence having secondary structure. Simply because splice sites are generic to different sequences that do not "convey" secondary structure that is needed for splicing does not mean that the mechanism does not encounter problems of unpredictability as taught by Balvay et al.

In response, as noted in the previous section, the discussion on pages 165-167 of Balvay et al. is couched in terms of differences in what would be expected from what was known at the time in terms of splice site selections. Without a set of rules that were considered to be generally predictive of splicing events, there would never be ”expectations” as well as exceptions and abnormality that disagreed with such expectations. Applicants further note that page 167 of Balvay et al. indeed addresses alternative splicing where secondary structure was invoked as a possible factor. It should be pointed out that although alternative splicing is a well known phenomenon, it is in and of itself an exception since most mRNA’s undergo a single splicing pattern.

Additionally, as noted above, evidence that the level of predictability for correctly inserting intron sequences is high is seen in other publications (e.g., Gatermann, Yoshimatu, Mayeda and Oshima). Applicants respectfully point out that in the recent Office Action, there were no comments that directly address this particular reference and how it bears upon the interpretation of Balvay. The teachings of Balvay can only be reconciled with the Gatermann, Yoshimatu and Mayeda and Oshima references if one assumes that most of the time, insertion will not be problematic but on occasion there may be sequences in an intron or exon that give results that are at odds with standard predictions.

The Office Action provides further comments with respect to Lewin:

> Furthermore, one or ordinary skill in the art would not be able to recognize which cells are "incompatible" or "compatible", as instantly recited, in view of the teachings of Lewin et al. that are cited by applicant. Specifically, if

> splicing sites are generic and do not have specificity for individual RNA precursors, as taught by Lewin et al., one would not be able to determine without undue experimentation how such introns would get excised from some cells and not from others, as instantly recited. The instant nucleic acid construct has to be able to allow excision of the intron in some cells but not in others.

Applicants, in response, respectfully point out that as stated in the response to the previous Office Action, one of skill in the art would be able to recognize compatible and incompatible cells. Splicing would not occur in incompatible cells but could occur in compatible cells. Furthermore, Applicants note that currently pending claims 245, 255 and 262 recites that the polymerase (claim 245) and the gene product (claims 255 and 262) is not expressed in the prokaryotic cell and is expressed in a eukaryotic cell.

The Office Action in the enablement rejection finally states:

> Furthermore, the replacement of even a few nucleotides on an mRNA can abolish all activity of the translated protein. It is maintained that neither the specification nor the prior art arms one of skill with the information necessary to engineer sequences into nucleic acid constructs that will be reliably spliced out to result in a protein with native activity restored.
>
> In order to practice the invention using the specification and the state of the prior art as outlined above, the quantity of experimentation required to practice the invention as claimed would therefore require the *de novo* determination of intervening sequences that can be fully spliced out without leaving behind any nucleotides that might interfere with native activity. In the absence of sufficient guidance from the specification, the amount of experimentation would be undue, and one would have been unable to practice the invention over the scope claimed.

Applicants, in response, again emphasize that success with intron insertion would be predictable to one of ordinary skill in the art. Furthermore, success with intron insertion generating predictable results was continued in publications that came out shortly after the priority date as exemplified in Johansen.

In conclusion, *contra* to assertions made in the Office Action, it is Applicants view that the state of the art at the time of the filing would have allowed a user to

proceed with insertion of introns with reasonable confidence that even in the new locations, the introns would most likely be properly spliced out and there would not be undue experimentation involved in either the choice of the intron or the choice of the insertion site that would allow suppression of expression of the gene product in a bacterial cell but would allow expression of a functional product in a eukaryotic cell. The insertion could be carried out using methods that were described in the specification or other methods that were described in the literature at the time of the filing.

Thus, in view of the above arguments, Applicants assert that the rejections under 35 USC 112, first paragraph (lack of enablement) have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

IV. The Rejections Under 35 USC 112, First Paragraph (Written Description-New Matter)

Claims 245-255, 258, 262 and 265 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The Office Action specifically notes that this particular rejection is a New Matter Rejection. In connection with this rejection, it is specifically stated:

> Claim 245 has been amended to recite that the polymerase is "incapable of being expressed in a prokaryotic cell" due to the presence of the intron and that the polymerase is capable of producing more than one copy of a nucleic acid sequence from the construct when introduced into a "eukaryotic" cell.
>
> Claim 255 has been amended to recite that the gene product is incapable of being expressed in a prokaryotic cell due to the presence of the intron and that the gene product or protein expressed from the gene product would be toxic specifically to a prokaryotic cell in the absence of the intron.
>
> Claim 262 has been amended to recite that the gene product is incapable of being expressed in a prokaryotic cell due to the presence of the intron, which in a eukaryotic cell is substantially removed during processing
>
> Each of these limitations constitutes new matter for the reasons explained below. Claims 246-254, 258, and 265

are rejected because they depend from claims 245, 255 or 262.

It is requested in the Office Action that support for each of the claim limitations in the specification be pointed out.

In response, Applicants first note that claim 245 has been further amended to recite that the nucleic acid construct which comprises a nucleic acid sequence which encodes a polymerase and comprises a recognition site for said polymerase and claims 255 and 262 which is directed to a construct containing a sequence that encodes a gene product and an intron sequence non-native to said gene product have been both further amended to recite that when the construct is in a eukaryotic cell, the intron is removed during processing and the gene product is expressed in a eukaryotic cell after removal of said intron.

Specific support for each of the independent claims is set forth in the table below:

Claim	Text	Support in the specification
245	A nucleic acid construct which comprises a nucleic acid sequence which encodes a polymerase	p.82, lines 4-5:"..A nucleic acid construct which when introduced into a cell express a non native polymerase"
		p. 82, lines 11-16"…The non native polymerase comprises a member selected from DNA polymerase, RNA polymerase and reverse transcriptase…The RNA polymerase preferably comprises a bacteriophage RNA polymerase, e.g., T3, T7, and SP6, or combinations thereof…"
		Example 19 (pages 145-155)
	and comprises a recognition site for said polymerase	Page 82, lines 7-8: "This construct can further comprise a recognition site for the non native polymerase".

	said construct further comprising an intron sequence, non-native to said polymerase, wherein said intron sequence is within the sequence encoding said polymerase	Page 89, lines 1-3: "In an application of the present invention, an intron is introduced into the coding sequence of T7 RNA polymerase in a construct that also contains a T7 promoter directing the transcription of a useful gene product.."
		Page 89, lines 8-11: "The present invention (see Examples) describes the conditional inactivation of a gene (that normally does no contain a processing element) by the precise introduction of an intron between the last two G's of a site that has the post splice junction sequence (C/A)AGG.
	and wherein said polymerase is (a) incapable of being expressed in a prokaryotic cell, due to the presence of said intron and	page 89, lines 13-16 "Therefore a construct with this modification should lack any expression of T7 RNA polymerase in an E. coli cell, but the normal coding sequence can be restored from transcripts after introduction into a compatible cell"
	(b) is capable of producing more than one copy of a nucleic acid sequence from said construct when introduced into a eukaryotic cell	page 82, lines 3-6: "…a nucleic acid construct which when introduced into a cell expresses a non native polymerase, the polymerase being capable of producing more than one copy of a nucleic acid sequence from the construct"

245		Page 157, lines 11-13: "After introduction into a eukaryotic cell, the RSV promoter directs the synthesis of the T7 RNA polymerase which in turns acts upon the T7 promoter to synthesize beta-galactosidase." describes the ability for the construct with the intron-modified T7 RNA polymerase to become functional in a eukaryotic cell and direct transcription describes the synthesis of more than one copy of beta-galactosidase.
255	A nucleic acid construct which comprises a sequence that encodes a gene product, said construct further comprising an intron sequence non-native to said gene product, wherein (a) said intron sequence is within the sequence encoding said gene product; (b) said gene product is incapable of being expressed in a prokaryotic cell due to the presence of said intron; and (c) said gene product would be toxic specifically to a prokaryotic cell in the absence of said non-native intron, which when in a eukaryotic cell, said intron is removed during processing and wherein said gene product is expressed in a eukaryotic cell after removal of said intron.	Page 81, lines 16-19: "The method utilizes the introduction of a heterologous processing element into the coding region of a desired gene resulting in inactivation of the gene when present in a non-compatible cell. The intron can be inserted at a number sites in most genes". Page 82, lines 1-3: "In a preferred embodiment, the gene product either is absent or inactive in an incompatible cell, but when introduced into a compatible cell yields a functional mRNA molecule which, upon translation, the gene yields an unaltered protein."

		Page 87, lines 4-14: "This invention has utility for the conditional inactivation of genes when such genes would be lethal to the host cell or when such genes present in a host cell introduce a danger…Genes coding for toxic products, including tetanus toxin, ricin, pseudomonas toxin, E. coli enterotoxins, cholera toxin and other plant, animal and microbial toxins can be inactivated and maintained stably and safely in an incompatible cell and activated to produce an unaltered gene product in a compatible cell".
262	A nucleic acid construct which comprises a nucleic acid sequence encoding a gene product and further comprises an intron sequence non-native to said gene product, wherein said intron sequence is inserted within a sequence encoding said gene product and	Page 81, lines 16-19: "The method utilize the introduction of a heterologous processing element into the coding region of a desired gene resulting in inactivation of the gene when present in a non-compatible cell. The intron can be inserted at a number sites in most genes".
	immediately 3' to (C/A)AG and said gene product is incapable of being expressed in a prokaryotic cell due to the presence of said intron, which when in a eukaryotic cell, said intron is removed during processing	Page 84, lines 6-12:" Rather than a restriction enzyme site, the frequently occurring sequence (C/A)AGG post splice junction sequence is used as the insertion site. This site results from the consensus sequence resulting from an excision of an intron. The consensus splice sequence or splice donors is (C/A)AG*GU and the consensus sequence for splice acceptors is (U/C)nN(C/U)AG*G where * represents the splice site…
	and wherein said gene product is expressed in a eukaryotic cell after removal of said intron.	Figure 25, particularly "Active T7 RNA polymerase is only made in eukaryotic cells after splicing out of SV40 intron"

Further assertions were made regarding support for claims 245, 255 and 262 in the Office Action. In particular, the Office Action on page states:

> The passage on page 85 discloses that in a prokaryotic environment, the intron should remain in the mRNA as long as a self-splicing intron is not used. This teaching does not support the instant amendments requiring for the polymerase to be incapable of being expressed in a prokaryotic cell due to the presence of the intron and being capable of producing more than one copy of a nucleic acid sequence when introduced into a eukaryotic cell; does not support the instant limitation that the gene product is incapable of being expressed in a prokaryotic cell due to the presence of the intron, which in a eukaryotic cell is substantially removed during processing; and does not support the limitation that the gene product is incapable of being expressed in a prokaryotic cell due to the presence of the intron, which in a eukaryotic cell is substantially removed during processing. The passage on page 85 simply discloses that it is possible that insertion of a heterologous processing element may not in all cases inactivate a gene when present in an "incompatible" cell and that in a prokaryotic environment, the intron should remain in the mRNA as long as a self-splicing intron is not used. However, the claims are not directed to whether or not self-splicing introns are utilized. Furthermore, simply because the specification discloses that non self splicing introns should remain in the mRNA in a prokaryotic environment, this teaching is not support for each of the instant limitations discussed above.

In response, Applicants assert that there is support for the above-stated claim limitations in the specification. As conceded in the Office Action, it is taught that in a prokaryotic environment (cell) an intron that is not “self-splicing” would remain in the mRNA (Pg 85). There is certainly further disclosure in the specification regarding the role of the intron. A description of one example of such an intron, the SV40 intron, is provided in Example 19 of the specification. Further, the effect of insertion of an intron is summed up on page 89 as

> Therefore a construct with this modification should lack any expression of T7 RNA polymerase in an *E. coli* cell, but the normal coding sequence can be restored from transcripts after introduction into a compatible cell.

This phrase would provide support at the very least for the limitation that the polymerase is incapable of being expressed in a prokaryotic cell due to the presence of the intron.

Events occurring within a "compatible" cell are discussed on pages 89-90 of the specification.

> In a compatible cell, normal expression of the product will occur but lethality should be negated by the nature of its environment. First, the autocatalytic cascade, due to transcription around the circular plasmid, believe to be responsible for the lethality in E. coli, would not occur in stably transformed mammalian cells .....

It can be seen that in this passage "mammalian cells" are being used as "compatible cells". At the end of Example 20 (page 157), the intron modified T7 RNA polymerase construct (pINT-3 made in Example 19) was used for directing synthesis of beta-galactosidase and the effects of the reconstituted T7 RNA polymerase coding sequence was described as follows: "After introduction into a eukaryotic cell, the RSV promoter directs the synthesis of the T7 RNA polymerase which in turns acts upon the T7 promoter to synthesize b-galactosidase." In this passage the term "eukaryotic cell" is being used in the context of a "compatible" cell.

Applicants further wish to clarify that the claims are not directed towards self-splicing introns. The specification as written, readily teaches away from the use of self-splicing introns since the functionality of these sequences is independent of cellular environment and as such, all cells are "compatible" and no types of biological cells would be considered "incompatible" for self-splicing intron. Thus the limitations of the claim are directed away from the use of self-splicing introns.

The Office Action further states

> Furthermore, Example 19 and the description of Example 19 at page 89 of the instant specification do not support the breadth of the instant limitations. Example 19 illustrates an example of the conditional activation of a gene by the precise introduction of an intron between the last two G's of a site that has the post splice junction

> sequence (C/A)AG, which is one example that is disclosed as being in hematopoietic cell lines, which are eukaryotic cells. This single example in a specific cell line with a construct with specific structural characteristics does not offer support for the broadly recited elements of the instant claims. This represents one species within a broad genus of nucleic acid constructs with broad structural features that are instantly claimed and not supported by the specification. The description of Example 19 discloses "Therefore, a construct with this modification could lack any expression of T7 RNA polymerase in an *E. coli* cell, but the normal coding sequence can be restored from transcripts after introduction into a compatible cell". Therefore, the specific example pointed to by applicant does not offer support for broad recitation of each of the structural characteristics of the nucleic acid construct discussed above and the specification does not support such broad recitation of polymerases that are incapable of being expressed in any prokaryotic cells due to the presence of an intron and are capable of producing more than one copy of a nucleic acid sequence when introduced into any eukaryotic cell; and does not support resultant toxicity to prokaryotic cells in general in the absence of the intron....

In response to the assertion that only one example of conditional inactivation of a gene is provided in Example 19, Applicants point out that 19 different (C/A)AGG sites were described as being located within the T7 coding sequence and "Any of these sites should be suitable" (pg 146). This statement is also consistent with the teachings of Mayeda and Oshima discussed above. Furthermore, there was extensive discussion of the problems of a plasmid that had both a polymerase and its cognate promoter on the same construct where it was considered to be a toxic product in a prokaryotic cell (Background of the Invention, pages 6-7) and it can be seen that both of the constructs described in Examples 19 and 20 have this configuration.

V. Summary and Conclusions

It is Applicants belief that the pending claims are in condition for allowance. However, if a telephone conversation would further the prosecution of the present

application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,

/Cheryl H Agris/

Dated: June 16, 2008

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